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Disruption of Thyroid Hormone Activation in Type 2 Deiodinase Knockout Mice Causes Obesity With Glucose Intolerance and Liver Steatosis Only at Thermoneutrality

Melany Castillo,¹ Jessica A. Hall,² Mayrin Correa-Medina,¹ Cintia Ueta,¹ Hye Won Kang,³ David E. Cohen,³ and Antonio C. Bianco¹

OBJECTIVE—Thyroid hormone accelerates energy expenditure; thus, hypothyroidism is intuitively associated with obesity. However, studies failed to establish such a connection. In brown adipose tissue (BAT), thyroid hormone activation via type 2 deiodinase (D2) is necessary for adaptive thermogenesis, such that mice lacking D2 (D2KO) exhibit an impaired thermogenic response to cold. Here we investigate whether the impaired thermogenesis of D2KO mice increases their susceptibility to obesity when placed on a high-fat diet.

RESEARCH DESIGN AND METHODS—To test this, D2KO mice were admitted to a comprehensive monitoring system acclimatized to room temperature (22°C) or thermoneutrality (30°C) and kept either on chow or high-fat diet for 60 days.

RESULTS—At 22°C, D2KO mice preferentially oxidize fat, have a similar sensitivity to diet-induced obesity, and are supertolerant to glucose. However, when thermal stress is eliminated at thermoneutrality (30°C), an opposite phenotype is encountered, one that includes obesity, glucose intolerance, and exacerbated hepatic steatosis. We suggest that a compensatory increase in BAT sympathetic activation of the D2KO mice masks metabolic repercussions that they would otherwise exhibit.

CONCLUSIONS—Thus, upon minimization of thermal stress, high-fat feeding reveals the defective capacity of D2KO mice for diet-induced thermogenesis, provoking a paradigm shift in the understanding of the role of the thyroid hormone in metabolism. *Diabetes* 60:1082–1089, 2011

Obesity results as the consequence of a positive energy balance, where energy intake is greater than energy expended. One of the key molecules in this balance is thyroid hormone, which potentially accelerates the resting energy expenditure (1,2). The adaptive (cold-induced) energy expenditure is controlled by the sympathetic nervous system and is also accelerated by thyroid hormone. In response to cold exposure, the sympathetic nervous system stimulates brown adipose tissue (BAT) and activates uncoupling protein 1 (UCP1) (3),

which is transcriptionally upregulated by thyroid hormone (4). In addition, the sympathetic nervous system also stimulates the cAMP-inducible type 2 deiodinase (D2) that amplifies thyroid hormone signaling in BAT by locally converting the prohormone T4 to the active form of thyroid hormone, T3 (5). Disruption of this pathway, as in mice with targeted inactivation of D2 (D2 knockout [D2KO] mice), leads to impaired BAT thermogenesis and hypothermia during cold exposure (6,7).

Sympathetic activity to BAT is also augmented by high-fat feeding (8), leading to diet-induced thermogenesis, but the role played by thyroid hormone in this process is largely unclear. Although there is an intuitive assumption that hypothyroid individuals/animals tend to be obese, the compilation of a vast array of data from individuals transitioning from hypo- to hyperthyroidism and vice versa exhibits only minor changes in body composition (9–11). In fact, we have reported earlier that hypothyroid rats living at room temperature placed on a high-fat diet do not accumulate more fat than euthyroid controls (12), questioning a role for thyroid hormone in this pathway.

However, it is conceivable that compensatory mechanisms activated during hypothyroidism may obscure the relevance (if any) of thyroid hormone on diet-induced thermogenesis. In this case, such mechanisms are likely to stem from the sympathetic nervous system, given that sympathetic activity fluctuates in an opposite direction as thyroid hormone signaling (13–15). In fact, the BAT-specific decrease in thyroid hormone signaling seen in the D2KO mouse is sufficient to trigger a compensatory increase in BAT sympathetic activity during cold exposure, upregulating a series of T3-responsive metabolic parameters in the tissue, including UCP1 mRNA levels (7).

Here, we report that even at room temperature there is a chronic increase in BAT sympathetic activity. We suggest that this activity compensates for the decreased thyroid hormone signaling, thus masking profound metabolic alterations in D2KO mice. If reared at 22°C, D2KO mice have increased tolerance to glucose and gain the same weight as controls on a high-fat diet. However, when the increase in BAT sympathetic activity is minimized by rearing animals at 30°C, D2KO mice develop intolerance to glucose and become more susceptible to diet-induced obesity. Remarkably, a consistent feature of the D2KO mice, independent of ambient temperature, is liver steatosis, which becomes most severe under high-fat feeding after acclimatization to thermoneutrality. Thus, these results provoke a paradigm shift in the understanding of the role of the thyroid hormone in metabolism, uncovering a hitherto unrecognized function for thyroid hormone in prevention of obesity and its metabolic complications.

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RESEARCH DESIGN AND METHODS

Animals. All studies were performed under a protocol approved by the local Institutional Animal Care and Use Committee. C57BL/6J and D2KO (7) mice approximately 3 months old were used from our established colonies, kept at room temperature (22°C), at thermoneutrality (30°C; Columbus Instruments, Columbus, OH), or in the cold (5°C), with a 12-h dark/light cycle starting at 0600 h, and housed in standard plastic cages with four male mice per cage. Animals were kept on standard chow diet (3.5 kcal/g; 28.8% protein, 58.5% carbohydrate, 12.7% fat) (5010 LabDiet laboratory autoclavable rodent diet; PMI Nutrition, Richmond, IN) or a high-fat diet (4.5 kcal/g; 15.3% protein, 42.7% carbohydrate, 42% fat) (TD 95121; Harlan Teklad, Indianapolis, IN) as indicated. Twenty-four-hour caloric intake was measured at the indicated times using the Oxymax Feed Scale device (Columbus Instruments). At the appropriate times, animals were killed with carbon dioxide. Tissue samples were obtained and immediately snap-frozen for further analyses.

Body composition. Lean body mass (LBM) and fat mass were determined by dual-energy X-ray absorptiometry (DEXA; Lunar Pixi, Janesville, WI). For the procedure, mice were anesthetized with ketamine-xylazine (200 mg/kg and 7–20 mg/kg) before imaging.

Indirect calorimetry. Oxygen consumption (V_{O_2}), respiratory exchange ratio (RER), and locomotor activity were continuously measured using the Oxymax System 4.93 (C.L.A.M.S.; Columbus Instruments). The animals were placed in the C.L.A.M.S. with free access to food and water, allowing them to acclimatize in individual metabolic cages for 48 h before any measurements. Subsequently, 24-h metabolic profiles were generated in successive 14-min cycles.

V_{O_2} was expressed as milliliters per kilogram LBM per minute. Studies were performed at 30°C, 22°C, or at 5°C for the indicated times. The sensor was calibrated against a standard gas mix containing defined quantities of O_2 and CO_2 . Spontaneous locomotor activity was recorded with OPTO-M3 Activity Application Device (Columbus Instruments) (16). All analyses for V_{O_2} and RQ were made considering the area under the curve (i.e., V_{O_2} vs. time; respiratory quotient [RQ] vs. time) for each individual animal.

Glucose tolerance test. Tolerance to a glucose load was studied in overnight fasted live mice following intraperitoneal injection of 1 g/kg glucose. Blood samples were obtained from the tail vein and measured with Glucometer Elite (Bayer Tarrytown, NY) at different time points.

mRNA analysis. Total RNA was extracted using the RNeasy kit (Qiagen, Valencia, CA) according to the manufacturer's instructions, quantified with a Nano-Drop spectrophotometer and 2.5 μg reverse-transcribed into cDNA by using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). Genes of interest were measured by quantitative RT-PCR (Bio-Rad iCycler iQ Real-Time PCR Detection System; Bio-Rad Laboratories Hercules, CA) using the iQ SYBR Green Supermix (Bio-Rad Laboratories) with the following conditions: 15 min at 94°C (Hot Start), 30–50 s at 94°C, 30–50 s at 55–60°C, and 45–60 s at 72°C for 40 cycles. A final extension at 72°C for 5 min was performed as well as the melting curve protocol to verify the specificity of the amplicon generation. Standard curves consisting of four to five points of serial dilution of mixed experimental and control groups cDNA were prepared for each assay. Cyclophilin A was used as a housekeeping internal control gene. The coefficient of correlation (r^2)

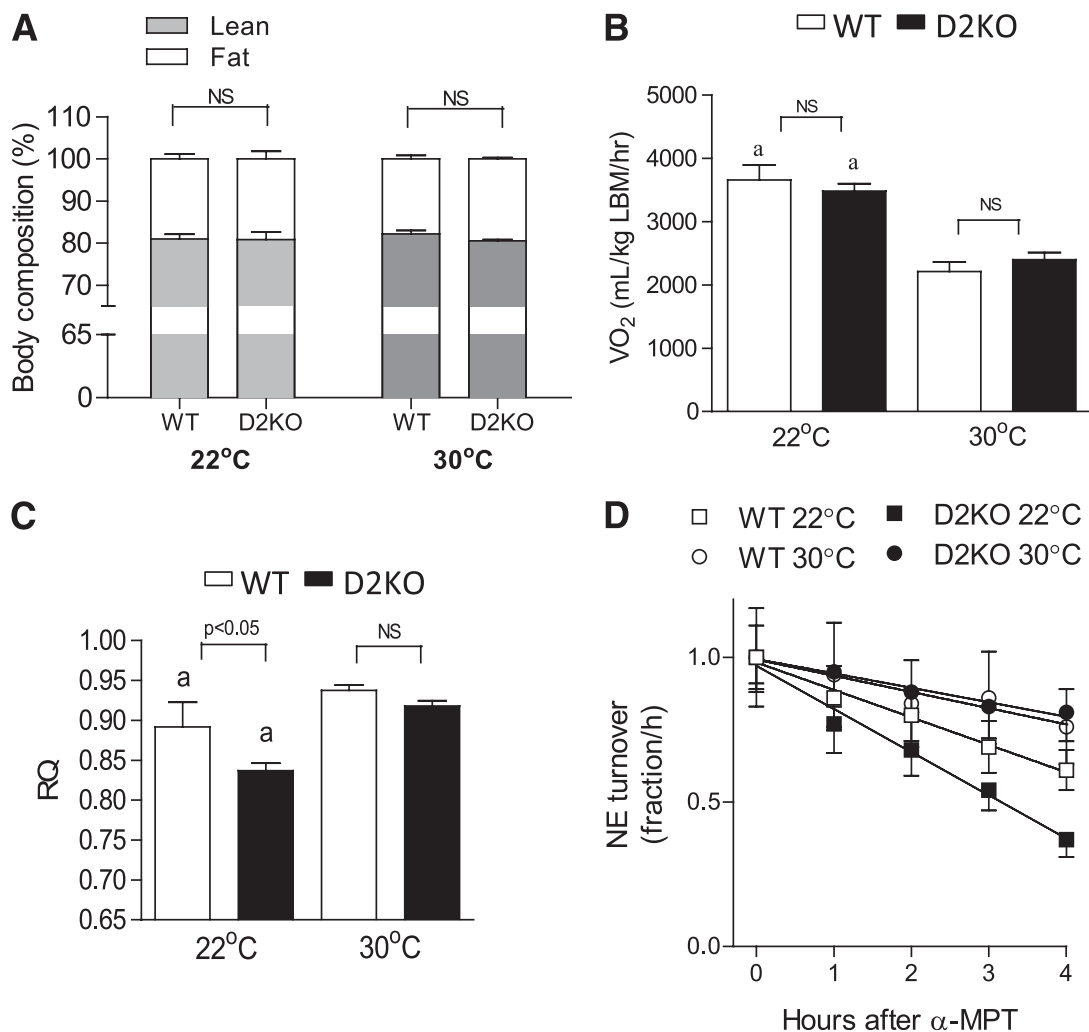


FIG. 1. Effect of ambient temperature on body composition, indirect calorimetry, and NE turnover of D2KO mice. **A:** Body composition as measured by DEXA in WT and D2KO mice acclimatized at the indicated ambient temperatures; body weights were as follows: D2KO, 21.55 ± 0.46 and WT, 25.4 ± 0.6 g at 22°C; D2KO, 22.4 ± 0.45 and WT, 23.9 ± 0.6 g at 30°C. **B:** Same as in **A**, except that what is shown is V_{O_2} . **C:** Same as in **B**, except that what is shown is RQ. **D:** Interscapular BAT NE turnover at the indicated time points. All animals were kept on chow diet. Measurements were made during the light cycle. Entries are means \pm SE of four to five animals; *a* is $P < 0.01$ vs. animals of the same genotype. NS, not significant.

was >0.98 for all standard curves, and the amplification efficiency varied between 80 and 110%. Results are expressed as ratios of test mRNA to cyclophilin A mRNA.

Interscapular BAT norepinephrine turnover. Interscapular BAT (IBAT) norepinephrine (NE) turnover was measured in mice acclimatized at room temperature (22°C) or at thermoneutrality (30°C). Mice were anesthetized with urethane (1.2 g/kg i.p.) and given 300 mg/kg α -methyl parathyrosine (α -MT) to block NE synthesis as described (7). Mice were killed at 0, 1, 2, 3, or 4 h after the α -MT injection, and the IBAT was processed for NE measurement by radioimmunoassay (Alpco Diagnostics, Windham, NH).

Biochemical analyses. Immediately after mice were killed, liver fragments were obtained and fixed in 4% paraformaldehyde in 0.1 mL PBS for 24 h at 4°C, frozen, sectioned, stained with Oil Red O, and counterstained with Meyer's hematoxylin. Frozen liver fragments (~200 mg) were homogenized, and lipids were extracted using chloroform/methanol (2:1) and 0.05% sulfuric acid as described (17). An aliquot of the organic phase was collected and mixed with chloroform containing 1% Triton X-100, dried under nitrogen stream, and resuspended in water. Triglycerides were determined using a commercially available kit (Sigma-Aldrich, St. Louis, MO).

Statistical analysis. All data were analyzed using Prism software (GraphPad Software, Inc., San Diego, CA) and are expressed as means \pm SE. One-way ANOVA was used to compare more than two groups, followed by the Newman-Keuls multiple comparison test to detect differences between groups. The Student *t* test was used to compare the differences between two groups. $P < 0.05$ was used to reject the null hypothesis.

RESULTS

D2KO mouse metabolic profile depends on ambient temperature. Under the mild thermal stress conditions of room temperature (22°C) and on a chow diet (12.7% fat),

D2KO mice have similar caloric intake (Supplementary Fig. 1A) and percent composition of lean and fat masses as age-matched wild-type (WT) controls (Fig. 1A). Even after 2 weeks of acclimatization at 30°C, percent body composition remains unchanged in chow-fed D2KO and WT mice (Fig. 1A).

We next analyzed parameters of energy homeostasis using indirect calorimetry. At 22°C, despite having a similar rate of oxygen consumption (VO_2 ; Fig. 1B), D2KO mice had a relatively higher percentage of fatty acid oxidation compared with WT, as reflected by a significantly lower respiratory exchange ratio (RQ; Fig. 1C). These findings led us to analyze the BAT NE turnover rate as an index of sympathetic stimulation of this tissue. Remarkably, although WT controls had a NE turnover rate of about $9.5 \pm 0.6\%/h$, D2KO animals maintained a rate of $\sim 15 \pm 1.1\%/h$ (Fig. 1D; $P < 0.01$).

Thus, to examine whether this difference in sympathetic activity depends on ambient temperature, D2KO and WT mice were acclimatized at 30°C. In this setting, the BAT NE turnover rate was reduced in both groups to $\sim 5\%/h$, with no differences between WT and D2KO mice (Fig. 1D). This was paralleled by a decrease in VO_2 as compared with the rates at 22°C, with D2KO mice maintaining similar values as WT mice (Fig. 1B). Of interest, thermoneutrality dissipated the differences in RQ between WT and D2KO mice (Fig. 1C), with RQ increasing significantly from 22°C values

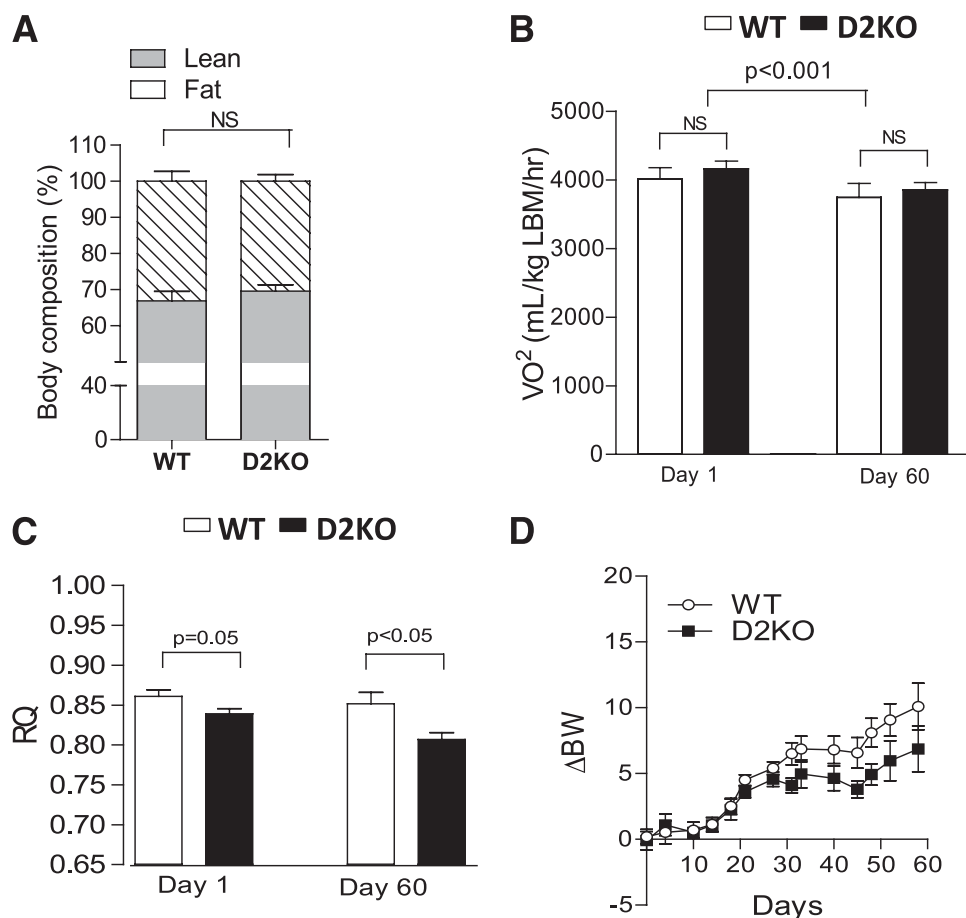


FIG. 2. Effect of high-fat feeding at room temperature on body composition and indirect calorimetry. D2KO and WT mice were fed with high-fat diet for 8 weeks and kept at 22°C (A–D). **A:** Body composition as measured by DEXA in WT and D2KO mice at the end of the experiment; body weights were D2KO, 26.9 ± 2.68 and WT, 36.3 ± 2.5 g. **B:** VO_2 was measured at day 1 and day 60 in WT and D2KO, after the animals started on the high-fat feeding. **C:** Same as B, except that what is shown is RQ. **D:** Body weight gain in WT and D2KO mice. Entries are means \pm SE of four to five animals; a is $P < 0.05$ vs. animals of the same genotype.

in both groups of mice (~ 0.9). In addition, at 30°C , no differences in food consumption between D2KO and WT mice were noted (Supplementary Fig. 1).

D2KO mice have similar weight gain on high-fat diet at room temperature. To test the sensitivity of D2KO mice to diet-induced obesity, groups of age-matched WT and D2KO mice maintained at 22°C were placed on a high-fat diet (42% fat). No major differences were found between WT and D2KO mice (Fig. 2). After 60 days of ad libitum high-fat diet feeding, both groups experienced a similar increase in body fat (from about 20–33%; $P < 0.01$) as reflected in the body composition analyses (Fig. 1A vs. Fig. 2A). After 60 days, there was a minimal decrease in VO_2 in both groups (Fig. 2B), with RQ remaining slightly lower in D2KO animals (Fig. 2C; $P < 0.05$). Despite similar caloric intake in both groups (Supplementary Fig. 1B), D2KO mice gained slightly less body weight as compared with WT (30 vs. 37%), although differences did not reach statistical significance (Fig. 2D; $P = 0.2$).

Thermoneutrality reveals sensitivity to diet-induced obesity in D2KO mice. To test the hypothesis that the increased metabolism at 22°C overrules the effect of hypothyroidism, we next repeated the 60-day feeding period with high-fat diet in WT and D2KO mice that were maintained at 30°C .

This time, major differences were indeed found between WT and D2KO mice (Fig. 3). After 60 days of ad libitum high-fat diet feeding, D2KO animals experienced a much greater increase in body fat (~ 20 –45%; $P < 0.01$) compared with WT animals (~ 20 –35%; $P < 0.01$) as reflected in the body composition analyses (Fig. 1A vs. Fig. 3A). There was a small but significant increase in VO_2 in both groups (Fig. 3B), but, most importantly, the difference in RQ was dissipated on the very day 1 of high-fat feeding (Fig. 3C). Although no differences in caloric intake were observed between D2KO and WT animals under these conditions (Supplementary Fig. 1C), D2KO mice had a 66% increase in body weight, which was nearly twice that of the 37% increase seen in WT mice (Fig. 3D; $P < 0.01$). The increased susceptibility of the D2KO mouse to obesity at 30°C could also be noted upon visual inspection (Fig. 3E). That the increased fat gain was attributable to defective diet-induced thermogenesis was supported by a $\sim 80\%$ lower *UCP1* expression in the BAT of D2KO mice (Fig. 3F).

D2KO exhibit liver steatosis and glucose intolerance. Histological and biochemical liver analysis revealed increased triglyceride deposits in D2KO mice that were kept on a chow diet at 22°C ($\sim 30\%$; Table 1; Fig. 4A and E). Acclimatization to 30°C did not significantly change fat deposition in liver of both groups of animals (Table 1;

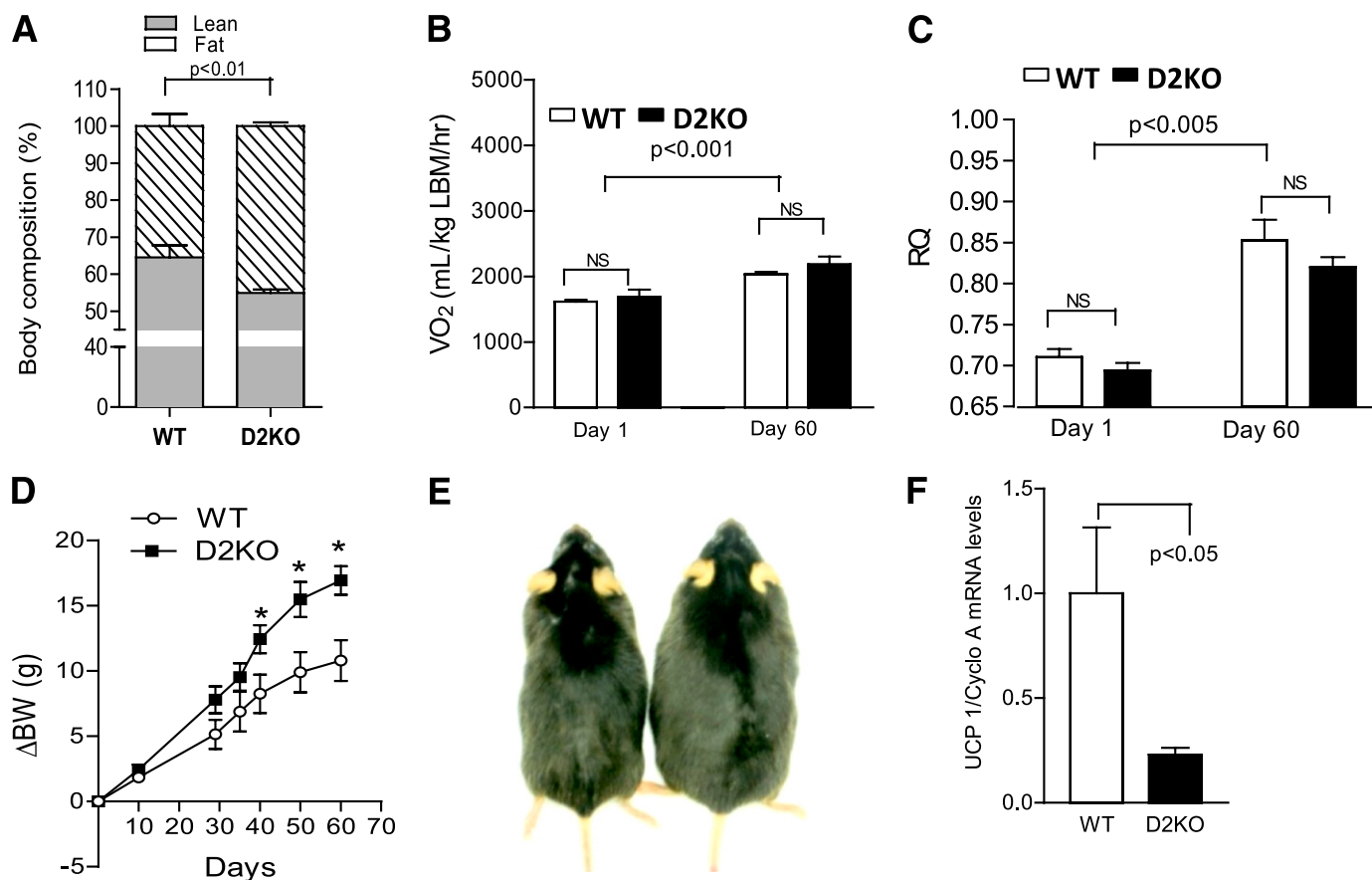


FIG. 3. Effect of high-fat feeding at thermoneutrality on body composition and indirect calorimetry. D2KO and WT mice were acclimatized at 30°C for 2 weeks and subsequently fed with high-fat diet for 8 weeks while at 30°C (A–F). A: Body composition as measured by DEXA in WT and D2KO mice at the end of the experiment; body weights were D2KO, 41.6 ± 1.23 and WT, 39.45 ± 1.8 g. B: VO_2 was measured at day 1 and day 60 in WT and D2KO. C: Same as B, except that what is shown is RQ. D: Body weight gain in WT and D2KO mice. At day 1 body weights were D2KO, 25.5 ± 0.57 and WT, 28.43 ± 1.12 g. E: Image of representative WT and D2KO mice at the end of the experiment. Entries are means \pm SE of four to five animals; * $P < 0.01$ vs. WT. (A high-quality color representation of this figure is available in the online issue.)

TABLE 1

Liver triglycerides content (in milligrams per gram) and serum NEFA levels (in milliequivalents per liter) in WT and D2KO mice kept on chow or high-fat diet: effect of environment temperature

Genotype	Liver triglycerides				Serum NEFA			
	Chow diet		High-fat diet		Chow diet		High-fat diet	
	22°C	30°C	22°C	30°C	22°C	30°C	22°C	30°C
WT	25 ± 0.7	47 ± 2.3	93 ± 2 ^b	126 ± 42 ^c	0.62 ± 0.06	0.3 ± 0.05 ^b	0.9 ± 0.06 ^b	0.66 ± 0.12 ^g
D2KO	33 ± 0.9*	58 ± 4.7 ^a	119 ± 5** ^e	339 ± 92** ^{d,f}	0.49 ± 0.06	0.6 ± 0.13*	0.9 ± 0.05 ^h	0.99 ± 0.04 ⁱ

All values in the table are means ± SE of four to five animals. * $P < 0.05$; ** $P < 0.005$; ^a $P < 0.057$ vs. WT on the same temperature and diet; ^b $P < 0.05$ vs. 22°C on chow diet; ^c $P < 0.01$ vs. 30°C on chow diet; ^d $P < 0.001$ vs. 22°C on high-fat diet; ^e $P < 0.001$ vs. 22°C on chow diet; ^f $P < 0.001$ vs. 30°C on high-fat diet; ^g $P < 0.05$ vs. 30°C on chow diet; ^h $P < 0.01$ vs. 22°C on chow diet; ⁱ $P < 0.01$ vs. 30°C on chow diet by one-way ANOVA.

Fig. 4B and F), and the differences between WT and D2KO were minimized (~22%; $P = 0.06$; Table 1; Fig. 4B and F). High-fat feeding for 60 days increased fat deposition in the WT liver by ~3.7-fold at 22°C and ~5.0-fold at 30°C (Table 1; Fig. 4C and D). In the D2KO livers, the increase in fat content reached ~3.6-fold at 22°C and, remarkably, 10-fold at 30°C (Table 1 and Fig. 4G and H). Although the differences of liver triglyceride content between WT and D2KO animals kept on a high-fat diet remained relatively stable at 22°C (~28%; Table 1 and Fig. 4C and G), moving the animals to 30°C dramatically increased this difference to ~2.7-fold (Table 1 and Fig. 4D and H).

The more extensive fat deposition in the liver of D2KO animals and its sensitivity to acclimatization temperature suggested that lipolysis and/or the level of serum fatty acids (nonesterified fatty acids [NEFA]) was playing a role. However, regardless of the diet, NEFA serum levels were not different between WT and D2KO animals at 22°C (Table 1). Of note, high-fat feeding did increase NEFA levels in both groups (Table 1). In addition, only WT animals experienced decreased NEFA serum levels when maintained at 30°C, which also does not correlate with our findings of fat deposition in the liver (Table 1). Thus, it does not seem that differences in serum NEFA levels contribute to liver steatosis in the D2KO animals.

Remarkably, the D2KO animals acclimatized at 22°C are substantially more tolerant to a glucose load than WT (Fig. 5A). Acclimatization to 30°C dissipated this difference in glucose handling (Fig. 5B), suggesting that chronic sympathetic BAT stimulation observed in D2KO mice at 22°C could make the animals more tolerant to glucose. This is supported by studies in rats, where cold exposure enhanced disposal of circulating glucose as a result of BAT activation (18). It is noteworthy that during high-fat feeding at 22°C, there were no differences between D2KO and WT animals in terms of glucose tolerance (Fig. 5C). During acclimatization at 30°C, feeding with a high-fat diet promoted glucose intolerance in D2KO mice, which were less capable of disposing of a glucose load (Fig. 5D).

DISCUSSION

Thyroid hormone and the sympathetic nervous system share a number of common target systems, including cellular pathways involved in metabolic control (1). BAT capitalizes on the synergistic relationship between the sympathetic and thyroid hormone systems for activation of adaptive thermogenesis. BAT expresses D2, which itself is a cAMP-responsive gene, increasing local T3 concentration four- to fivefold during sympathetic stimulation without significant alteration of systemic T3 levels (19); the end

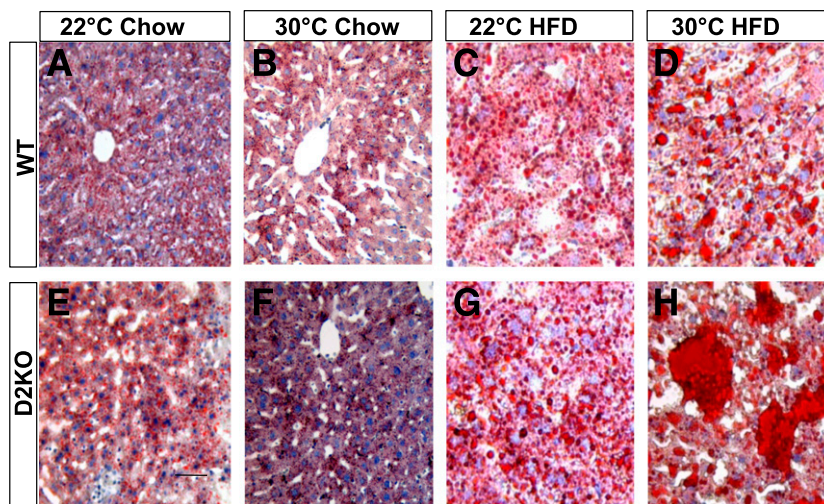


FIG. 4. Effect of acclimatization temperature and/or diet on lipid deposition in the liver. Oil Red O staining of liver sections obtained from D2KO and WT fed with chow or high-fat diet (HFD) for 8 weeks, acclimatized to 22°C or 30°C, as indicated (A–H) is shown. A and B: D2KO and WT fed with chow diet, acclimatized to 22°C. C and D: Same as A and B, except acclimatization was at 30°C. E and F: D2KO and WT fed with high-fat diet for 8 weeks, acclimatized to 22°C. G and H: Same as E and F, except acclimatization was at 30°C. Scale bar is 50 μ m. (A high-quality digital representation of this figure is available in the online issue.)

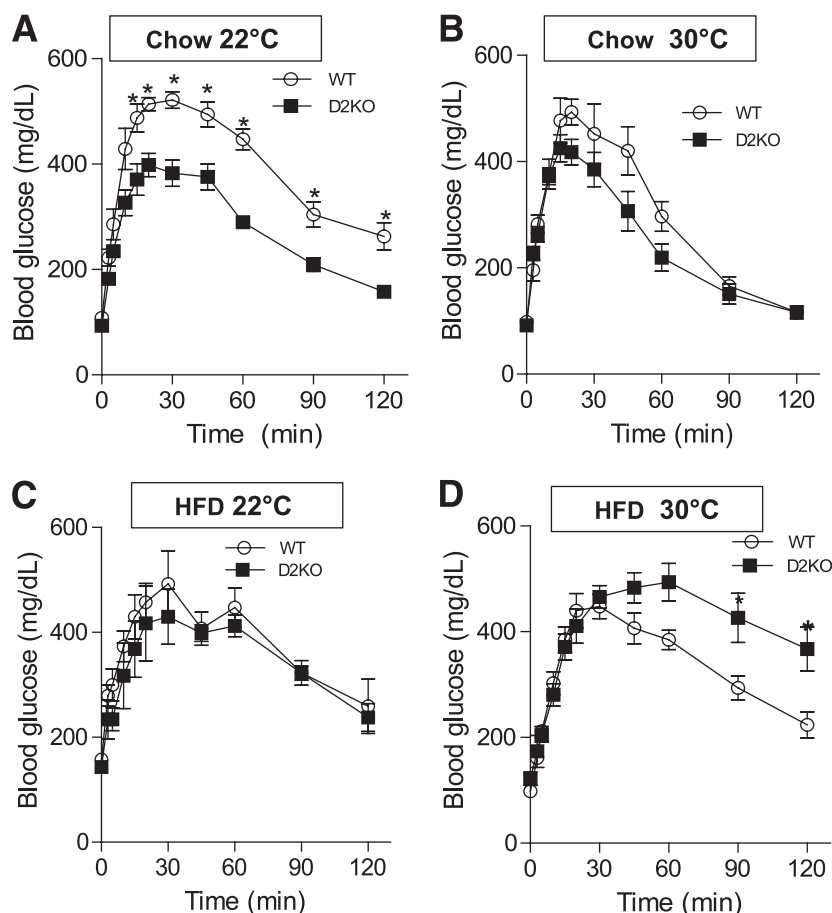


FIG. 5. Effect of temperature and/or diet on glucose tolerance. Blood glucose concentrations at the indicated time points following intraperitoneal injection of 1 g/kg glucose in D2KO and WT animals fed with chow or high-fat diet, acclimatized to 22°C or 30°C, as indicated are shown. **A:** D2KO and WT fed with chow diet, acclimatized to 22°C. **B:** Same as **A**, except acclimatization was at 30°C. **C:** D2KO and WT fed with high-fat diet for 8 weeks, acclimatized to 22°C. **D:** Same as **C**, except acclimatization was at 30°C. Entries are means \pm SE of four to five animals; * P < 0.01 vs. WT.

result being upregulation of T3-dependent genes such as UCP1, which is both cAMP- and T3-sensitive (5,20). Here, we show that when D2-mediated T3 production is prevented, as with the D2KO mouse, there is a compensatory increase in BAT sympathetic activity to offset the tissue-level hypothyroidism (Fig. 1D). We suggest that this compensatory sympathetic response neutralizes much of the phenotype that D2KO mice would otherwise exhibit as a result of the disruption in thyroid hormone signaling (Figs. 1 and 2). At 22°C, the D2KO mouse preferentially oxidizes fat (Fig. 1C), has a similar sensitivity to diet-induced obesity (Fig. 2), and is supertolerant to a glucose load (Fig. 5A). However, by eliminating thermal stress and rearing these animals at thermoneutrality (30°C), an opposite phenotype is encountered, one that includes obesity (Fig. 3) and glucose intolerance (Fig. 5D). These results define a critical role played by D2 in adaptive thermogenesis, revealing a novel aspect of the thyroid-adrenergic synergism.

Uncoupling substrate oxidation from ATP synthesis is an important pathway for maintaining body temperature when small mammals are exposed to cold. Given recruitment of BAT and increased adrenergic responsiveness in mice fed a cafeteria diet (21), a similar pathway may be harnessed to activate BAT and dissipate excess calories as a form of diet-induced thermogenesis. In fact, activation of the adrenergic system has been used to counteract obesity (22). However, similar to our present findings, studies

performed with high-fat feeding of *UCP1*^{-/-} mice, which were expected to become obese, yielded drastically different phenotypes that were dependent on whether ambient temperature prevented or promoted thermal stress. Although *UCP1*^{-/-} mice reared at 30°C are prone to diet-induced obesity, under subthermoneutral temperatures, *UCP1*^{-/-} mice are lean with elevated D2 in inguinal fat (23,24). It has been suggested that in the absence of the UCP1 pathway, alternative mechanisms are triggered to maintain body temperature, such as an increase in thyroid hormone signaling. Consequently, when both UCP1 and a thyroid hormone-responsive mechanism, glycerol phosphate cycling, are inactivated, mice accumulate even less fat mass at 22°C (25). Our current findings support and confirm this notion of the importance of thyroid hormone (and its activation by D2) as an efficient means for maintenance of thermal homeostasis, where compromising the action of thyroid hormone leads to obesity only when without a thermal challenge.

So far, the link between thyroid hormone and body weight has been anecdotal. Although patients and lay individuals almost immediately associate hypothyroidism with obesity, the incidence of hypothyroidism in obese individuals is not increased, and changes in body composition during the transition from severe hypothyroidism to mild thyrotoxicosis are meager (9–11). Given our data, the mild apparent impact of thyroid dysfunction on metabolism

is likely the result of the effectiveness of the sympathetic-mediated compensatory mechanisms, whereby an inverse relationship exists between T3 and sympathetic signaling. By inactivating the sympathetic system through acclimatization to 30°C, we could better appreciate the importance for thyroid hormone activation on metabolic control, i.e., weight gain, tolerance to glucose load, and liver steatosis (Fig. 3). Thus, if this hypothesis proves to be correct, it is likely that a failure to trigger these strong compensatory mechanisms would result in symptoms and signs that would be more in line with our intuitive reasoning and prove clinically relevant. This is particularly pertinent given the finding of substantial amounts of functional BAT in adult humans (26).

Liver steatosis is a novel aspect of the D2KO phenotype (Fig. 4), which could be explained by increased NEFA uptake, impaired β -oxidation, and/or decreased secretion of VLDL. The lower RQ in the D2KO suggests that increased NEFA is a contributing factor, but no correlation could be found in the WT or D2KO animals between NEFA levels and liver steatosis (Table 1). Thyroid hormone is known to induce peroxisome proliferator-activated receptor (PPAR) (27) and β -oxidation in the liver (28) and, in BAT, D2 is a downstream target of FGF21 through a PPAR-mediated mechanism (29). In addition, liver delivers triglycerides to peripheral tissues by production of VLDL, of which apolipoprotein B, which is positively regulated by thyroid hormone (in HepG2 cells), is a major component (30). Given that serum T3 levels are normal in the D2KO mouse (31), it is conceivable that the D2 pathway is locally controlling thyroid hormone activation in liver and loss of which is directly contributing to the liver phenotype in the D2KO mouse. Although liver is known as a D1-expressing tissue, we have found measurable liver D2 activity and mRNA that are induced many fold and play a role in the double LXR KO mouse phenotype (32). It is noteworthy that hypoxia, a known inducer of the type 3 deiodinase (D3), which inactivates thyroid hormone and creates local hypothyroidism, aggravates liver steatosis and inhibits PPAR expression (33). Thus, it is conceivable that an active D2 pathway in liver upregulates genes involved in fatty acid economy. In addition, given the wealth of information about cross-talk between the sympathetic nervous system and the liver (34), it is also conceivable that brain D2 plays an indirect metabolic role in the liver via its expression in the medial basal hypothalamus and/or other brain regions.

Although it can be debated whether diet-induced thermogenesis exists and whether it exists outside of UCP1 and BAT, undoubtedly, adrenergic signaling plays a key role in the development of or protection from obesity upon disruption of thyroid hormone signaling. It is possible that variations in adrenergic signaling and not BAT itself could explain the energy balance in D2KO mice. Increased susceptibility to obesity at 30°C may be because of decreased adrenergic signaling and not the role of D2 in diet-induced thermogenesis per se, as has been suggested for *UCP1*^{-/-} mice (35). It also has been recently suggested that diet-induced thermogenesis takes place in tissues other than BAT, such as muscle (36). D2 is highly expressed in BAT, and, thus, it is logical to assume that the present results are directly related to the action of D2 in this tissue. However, we have not looked directly at oxygen consumption of BAT, so diet-induced thermogenesis could stem from elsewhere. D2 is expressed in a number of other tissues, including skeletal muscle (37), and the contribution of D2 in these tissues to metabolic control remains to be

elucidated. Finally, it could be argued that it is the mere fact that the D2KO mice are at 22°C (and thus have an increased metabolism) that hides the true phenotype observable at 30°C. In this case, there would be no compensatory mechanisms, i.e., the increased metabolism at 22°C would simply override any other effects.

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